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Role of arbuscular mycorrhizal fungi in phytoremediation of heavy metals and effects on growth and biochemical activities of wheat (*Triticum aestivum* L.) plants in Zn contaminated soils

Sadia Kanwal*, Asma Bano and Riffat Naseem Malik

Environmental Biology and Ecotoxicology Laboratory, Department of Environmental Sciences, Faculty of Biological Sciences, Quaid-i-Azam University, Islamabad, Pakistan.

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The increase of metals pollution in soil is a worldwide problem that affects the health of humans and environment. The use of green technology such as phytoremediation is one of the environmental friendly techniques, in which plants and other microbes are used to reduce the level of metals contaminants in soil and lower its uptake towards plants tissues. Studies report that a number of cereal crops such as wheat accumulates heavy metals in their tissues at higher concentrations. In the present study, we investigated the effect of arbuscular mycorrhizal fungi (AMF) on wheat plants with the increase of three different zinc (Zn) concentrations (0, 100, 300 and 900 mgkg^{-1}) in soil. After eight weeks of pot experiment, roots colonization, shoot and root biomass, growth, heavy metals contents and other biochemical parameters were assessed. The results indicate mycorrhizal inoculated (M) plants performed better at moderate Zn concentrations (300 mgkg^{-1}). In AMF associated plants, Zn contents were lower in shoot part of plants as compared to roots. In addition, higher P contents were observed in M treated plants as compared to NM plants. The decrease of nutrient contents, growth and antioxidant enzymatic activities were found at the highest applied Zn concentrations (900 mgkg^{-1}). Results indicate that AMF inoculum exhibit different tolerance strategies to reduce metals toxicity in host plants. The effective mycorrhizal symbiosis was observed with wheat plants and can be useful for phytostabilization of Zn contaminated soils which can play a vital role in the increase of food productivity and safety.

Key words: Wheat, arbuscular mycorrhizal fungi, phosphorus, nutrient contents, antioxidant enzymes.

INTRODUCTION

Soil contamination with heavy metals is the most challenging problem nowadays that is the consequence of increasing emissions from both natural and anthropogenic sources. The increase in the level of

*Corresponding author. E-mail: skkanwal7@gmail.com.

metals threatens the quality of food and management of sustainable environment (Thounaojam et al., 2012). Among the metals, Zn is one of the essential micronutrients required by plants in adequate quantities and plays an important role in numerous structural and metabolic processes of plants. Excessive concentrations of Zn in soil increase the plants toxicity consequently decrease the plant growth, yield and level of reactive oxygen species (ROS) (Sgherri et al., 2002).

Nowadays, the useful function of mycorrhiza in decrease of phytotoxicity has been generally known. The mechanism of phytoremediation can be increased by using arbuscular mycorrhizal fungi (AMF) in soil. AMF are symbionts colonized with plant roots found in many plant species and contaminated soils with heavy metals. AMF immobilize Zn in plant roots which decrease the harmful effects of metals on plants physiological and metabolic functions. Several studies indicated that plants grown in the presence of AMF have developed a variety of useful mechanisms that reduce the metals uptake in plants, increase the nutrient contents and form an efficient symbiotic relationship by inducing several mycorrhizal structures inside plant roots like arbuscules or vesicles (Gohre and Paszkowski, 2006).

Despite the importance of wheat crop in agriculture, no detailed information is available about biochemical alterations induced in the crop due to excessive metals concentrations (Mallick et al., 2011). In addition, little information is available with respect to association of mycorrhiza with wheat plants under toxic Zn concentrations, and the influence of mycorrhization on physiological and biochemical functions of wheat. The purpose of the study was to investigate the effects of mycorrhizal inoculation on growth, nutrients and biochemical contents of wheat plants grown in low (100 $mgkg^{-1}$), moderate (300 $mgkg^{-1}$) and high (900 $mgkg^{-1}$) Zn concentrations. The hypothesis of the study was that the use of AMF inoculum in metals contaminated soils immobilizes the metals contents in plant roots, increase growth, nutrients contents and other biochemical activities in plant tissues which enhance the quality and food productivity.

METHODOLOGY

Experimental design

The pot experiment was conducted under controlled laboratory conditions. The treatments consisted of (a) AMF inoculated wheat plants and (b) non-inoculated plants; with different applied Zn concentrations (0, 100, 300 and 900 $mgkg^{-1}$) in soil. All the treatments were installed in replicates, consisting of six replicates for each treatment, with the total of 48 pots.

Soil preparation

Soil and sand were collected to install the experiment from the vicinity of Quaid-i-Azam University, Islamabad, Pakistan. The average temperature of the area was 20 to 25°C. The soil and sand samples were air-dried, and sieved with 2 mm sieve to remove any debris from the samples. The chemical analysis of soil samples was carried out before the start of experiment. Soil was chemically characterized with a pH (6.7), T. Phosphorus (4.3 $mgkg^{-1}$), T. Potassium (19.5 $mgkg^{-1}$), Calcium (34.45 $mgkg^{-1}$), Magnesium (42.50 $mgkg^{-1}$), Extractable nitrate nitrogen (1.04 $mgkg^{-1}$), Extractable potassium (1.45 $mgkg^{-1}$), Extractable phosphorus (1.53 $mgkg^{-1}$), Zinc (1.50 $mgkg^{-1}$), Nickel (1.33 $mgkg^{-1}$), Copper (30.3 $mgkg^{-1}$), Cadmium (1.60 $mgkg^{-1}$), Iron (28.51 $mgkg^{-1}$), Lead (1.6 $mgkg^{-1}$), Chromium (4.25 $mgkg^{-1}$) and Manganese (10.4 $mgkg^{-1}$) respectively. Then, soil and sand were autoclaved twice for 1 h at 120°C to remove any local AMF spores. After autoclaving, soil and sand were combined with the ratio of 1:3 and used in pots to grow the wheat plants. Plants were treated with low (100), moderate (300) and high (900) concentrations of Zn ($mgkg^{-1}$) in soil.

Use of AMF Glomus species

Glomus species were used as AMF fungal inoculum in the pot experiment and mixed with the soil. The inoculum was obtained from the company in France. The two pot experiments with wheat plants were installed; (1) with the application of AMF fungal inoculum and applied Zn concentrations (0, 100, 300 and 900 $mgkg^{-1}$) in soil; (2) with the application of different Zn concentrations (0, 100, 300, 900 $mgkg^{-1}$) in soil. About 50 g of AMF inoculum were used in mycorrhizal (M) associated wheat experiment 1 (mycorrhizal experiment). There was no inoculation of mycorrhizal spores in the second experiment [non-mycorrhizal (NM) experiment].

Disinfection and germination of wheat seeds

Seeds of wheat (*Triticum aestivum* L.) were taken from Department of Crop Science, National Agriculture Research Centre, Islamabad. Seeds were sterilized by using Clorox, and soaked for about 10 min. After sterilization, seeds were appropriately washed with Milli-Q water several times and then placed on Petri dishes in dark (for about 2 days) for germination.

Seeds sowing and harvesting of plants

After seed germination, seedlings were sown in pots and grown for 8 weeks in a greenhouse with 16 h daylight (20 to 22°C). About 5 seedlings were grown in each pot. Proper light (12 h) and temperature conditions (25 to 30°C) and water conditions (on daily basis) were maintained during the experiment. Ashton nutrient solution (10 mL) was provided to plants once in a week. There were about 6 replicates for each treatment. Plants were harvested after 8 weeks of growing in pots.

Mycorrhizal colonization of roots

After harvesting of plants, roots and shoots were separated. Fresh roots samples were used for assessment of mycorrhizal colonization and line intersect method was used to evaluate the percentage of colonization (Giovannetti and Mosse, 1980). For each treatment, mycorrhizal colonization percentage was calculated.

Biomass of plants

Plants roots and shoots were washed with tap water to remove any

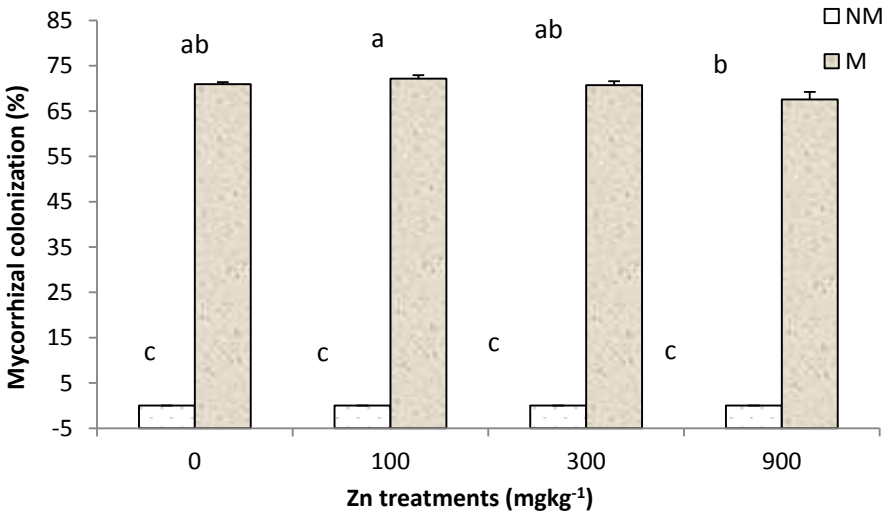

Figure 1. *Arbuscular mycorrhizal* fungi (AMF) colonization percentage with roots of wheat (*Triticum aestivum*) plants under increasing Zn concentrations. Data are presented as means ± SE of the mean. Bars represent standard error. The different letters above the bars shows significant difference between treatments according to Tukey test *(P<0.05*).

soil particles. Then, fresh weights of plants tissues were measured. After measurement of fresh weight, samples were placed in the oven for drying and then dry weight was determined. Biomass of plants tissues were assessed by calculating the difference between fresh weight and dry weight of the samples.

Plant growth

Shoot and root growth in M and NM plants were measured separately. Growth was measured by recording parameters including length, breadth and area of the plants samples. Vernier caliper (ECV150C, China) was used to measure the length and diameter of plant tissues.

Metal contents in plants

Plants tissues (shoots and roots) were analyzed separately for macro and micronutrient contents. Samples were dried, ground, digested in HNO_3 and H_2O_2 by using microwave digestion system (CEM-MDS 2000). After digestion, plants shoot and roots were analyzed for metals contents including macro (Mg, K, Na, Ca) and micro-nutrient contents (Cr, Cu, Ni, Mn, Zn, Pb, Fe, Cd) by using atomic absorption spectrophotometer (Varian FAAS-240). Plant tissues were also analyzed for total phosphorus (Ryan et al., 2001) and nitrogen contents (Van and Walinge, 1973).

Biochemical contents of plants

Fresh leaves of wheat plants were assessed for total chlorophyll, carotene (Duxbury and Yentsch, 1956), proline (Bates et al., 1973), sugar (Dubois et al., 1956) and protein contents (Lowry et al., 1951).

Antioxidant enzymes activity in plants

Fresh samples of plants were analyzed for antioxidant enzymatic activities. Superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), and ascorbate peroxidase (APX) activities were analyzed by using spectrophotometer. SOD in fresh leaves was analyzed by using the method of Beauchamp and Fridovich (1973). POD activity was evaluated by using the procedure of Gorin and Heidema (1976). CAT and APX activity were determined by using the method of Goel and Sheoran (2003) and Nakano and Asada (1981).

Quality control (QC/QA) analysis

Quality of analysis were measured by performing analysis in replicates (n=6) and by repeated measurements of each sample. To verify the accuracy of procedures, standard reference soil (NIST, 2709 San Joaquin) and plant materials (NIST, 1547 Peach leave) of National Institute of Science and Technology were included in each sample batch. Metals recovery was about 99%. After measurement of five samples, blank samples were run.

Statistical analysis

All the parameters (mycorrhizal colonization, metals contents, growth parameters, biochemical contents, antioxidant enzyme activities) in M and NM treatments were analyzed by two way analysis of variance (ANOVA) using statistix (version 8.1) software. In all treatments and analysis, Tukey test was used for comparison of means at 5% level.

RESULTS

Roots mycorrhizal colonization

Figure 1 shows the colonization of mycorrhizal structures with plants roots. No colonization was observed in NM treatments. The AMF plants showed higher percentage of colonization in all treatments. The presence of M colonization with plant roots indicated the existence of

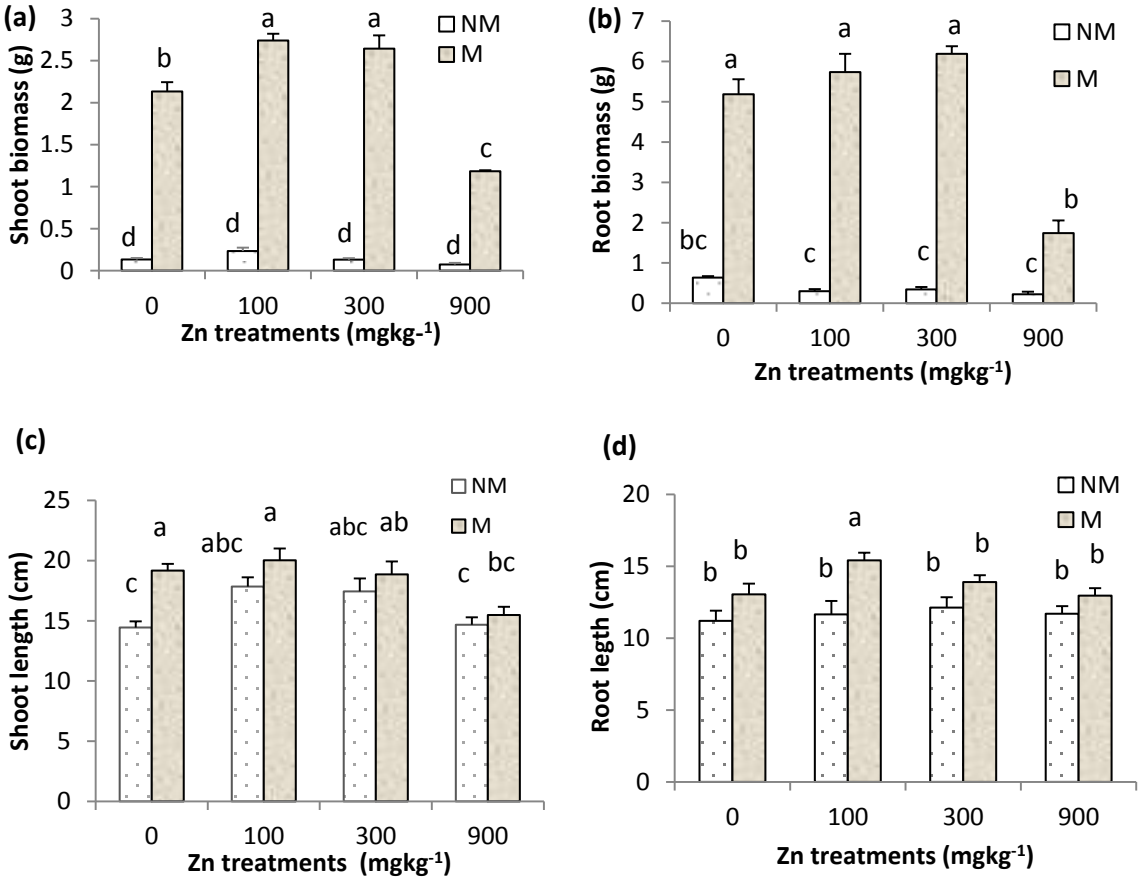

Figure 2. Effect of increasing zinc concentrations on wheat growth in mycorrhizal and non mycorrhizal treatments. **(a. b)** Plant shoot and root biomass. **(c. d)** Plant shoot and root length. The data shown are the means and standard error. The different letters above the bars indicates significant difference by Tukey test ($P < 0.05$).

mycorrhizal structures (arbuscules and hyphae). From the results, it is clear that the symbiotic relationship between wheat and mycorrhizal inoculum can be established under higher zinc concentrations. It is observed from the results that highest percentage of M colonization (72.16%) was observed at lowest Zn applied concentration (100 mgkg^{-1}), while the lowest M colonization (67.56%) was observed at highest applied Zn concentration (900 mgkg^{-1}). The results indicated that excessive Zn concentrations in soil negatively effects mycorrhizal colonization of plants roots.

Plant growth and biomass

The effect of increasing Zn concentrations (100, 300, 900 mgkg^{-1}) on physiological parameters (length, breadth, area) were examined in M and NM associated wheat plants (Figure 2). It was observed from the results of present study that M inoculation had positive effects on growth and biomass of wheat plants under increasing Zn concentrations as compared to NM wheat plants. Figure 2a and b indicates that AMF associated wheat plants showed significantly higher shoots and root biomass as compared to plants not inoculated with AMF. This was also observed from the results that excessive Zn concentrations badly affect the wheat plants in both M and NM plants. In 100 and and 300 mgkg^{-1} soil, a significant increase ($P < 0.05$) was noticed in shoot and root biomass of mycorrhizal associated plants, while lower trend was observed at highest applied Zn concentration (900 mgkg^{-1}).

Figure 2c to d shows that increasing concentrations of Zn affected the length of plants tissues in M and NM inoculated plants. Results show that high Zn concentration (900 mgkg^{-1}) caused reduction of both shoots and root growth in M and NM plants. However, significant increase in growth was observed in M associated wheat plants at 100 and 300 mgkg^{-1} Zn concentrations. Overall, higher shoot and root length was observed in M wheat plants as compared to NM plants at lower and moderate Zn applied concentrations. Therefore, all the results show that mycorrhizal associated plants showed better growth at Zn concentrations (0, 100 and 300 mgkg^{-1}) in soil. However, at maximum Zn concentration (900 mgkg^{-1}), reduction in

Table 1. Concentration of macro-nutrients measured in shoots and roots of mycorrhizal (M) and non-mycorrhizal (NM) wheat plants grown in soils with increasing Zn concentrations.

Experiment (Zn, mgkg^{-1})		K (gkg $^{-1}$)	P (gkg $^{-1}$)	N (gkg $^{-1}$)	Ca (gkg $^{-1}$)	Mg (gkg $^{-1}$)	Na (gkg $^{-1}$)
Shoot							
0	NM	24.927±2.6509^{ab}	0.4200±0.0551^{b}	0.2567±0.0233^{cd}	16.563±1.8148^{a}	10.620±0.6526^{ab}	18.523±0.6479^{bc}
	M	30.437±5.1117^{a}	1.3633±0.2469^{a}	0.5167±0.0273^{bc}	12.820±0.7240^{a}	8.4767±0.7656^{ab}	22.507±1.0287^{ab}
100	NM	19.073±2.3507^{ab}	0.3867±0.0606^{b}	0.2800±0.0208^{cd}	14.657±1.8712^{a}	11.800±1.0601^{a}	18.387±1.3594^{bc}
	M	26.520±2.6951^{ab}	1.2867±0.1695^{a}	0.8033±0.1257^{ab}	14.943±1.0199^{a}	10.653±0.8045^{ab}	23.133±1.4546^{ab}
300	NM	21.657±1.2451^{ab}	0.4000±0.0265^{b}	0.2400±0.0379^{cd}	14.310±0.5024^{a}	9.2900±0.4789^{ab}	15.613±1.6034^{cd}
	M	27.997±1.4801^{ab}	1.4033±0.2338^{a}	0.8467±0.0762^{a}	15.167±0.7503^{a}	9.2800±0.8253^{ab}	25.547±2.1620^{a}
900	NM	16.073±1.4694^{b}	0.7367±0.0906^{ab}	0.2167±0.0328^{d}	11.280±1.0660^{a}	7.7767±1.1893^{b}	11.510±0.5541^{d}
	M	17.697±0.5622^{b}	0.8700±0.1270^{b}	0.6267±0.0491^{ab}	12.363±1.3879^{a}	8.7300±0.3912^{ab}	14.140±0.9361^{cd}
Roots							
0	NM	10.807±0.8940^{c}	0.2600±0.0473^{c}	0.1643±0.0160^{bc}	2.8967±0.3325^{bc}	4.4767±0.6196^{ab}	7.4067±0.5881^{b}
	M	16.270±1.7304^{abc}	0.8433±0.2106^{a}	0.2800±0.0265^{ab}	4.4233±0.4943^{ab}	5.9467±0.3645^{a}	9.8267±0.1393^{a}
100	NM	13.467±0.5957^{bc}	0.3567±0.0491^{bc}	0.1700±0.0140^{bc}	3.0967±0.2601^{bc}	4.8267±0.4157^{ab}	7.7100±0.3499^{b}
	M	11.293±0.5877^{c}	0.7700±0.0208^{ab}	0.3200±0.0346^{a}	4.7500±0.4200^{a}	6.1833±0.4366^{a}	9.9167±0.1690^{a}
300	NM	18.547±0.7647^{ab}	0.3767±0.0546^{bc}	0.1167±0.0203^{c}	2.5233±0.2981^{c}	4.9533±0.0410^{ab}	7.3267±0.3018^{bc}
	M	16.177±0.5700^{abc}	0.7200±0.0681^{ab}	0.3433±0.0291^{a}	4.3567±0.3524^{ab}	5.2933±0.2256^{ab}	8.1733±0.2700^{b}
900	NM	17.737±1.2603^{ab}	0.4533±0.0809^{abc}	0.0700±0.0153^{c}	2.6633±0.2226^{c}	3.3233±0.4997^{b}	4.7333±0.3467^{d}
	M	19.533±1.7776^{a}	0.6533±0.0788^{abc}	0.2600±0.0289^{ab}	2.9900±0.1779^{bc}	3.4800±0.3365^{b}	5.7500±0.1762^{cd}

Data are presented as mean ± SD (n = 3) and have been analyzed by two way analysis of variance. Means followed by the same letter within columns are not significantly different by Tukey's test at the 5% level.

plant growth was observed in both M and NM associated plants.

Macro and micro-nutrients uptake

Tables 1 and 2 shows macro-nutrients and micro-nutrients contents of wheat plants (shoot and root) in both M and NM treatments with increasing Zn concentrations. Overall, in M associated treatments, improved nutrients contents (N, P, K, Ca, Mg, Na, Fe, Cu) were recorded. However, significant variations were noticed for nutrients uptake (K, P, Na, N, Cu and Mn) in both M and NM plants. However, non-significant results were observed for Ca and Mg at each Zn concentration. Excessive Zn addition level (900 mg kg^{-1}) had a harmful effect on nutrients uptake, as there was a significant decline of nutrients contents observed in both M and NM treatments.

The results of the experiment reveal that mycorrhization significantly affects the nutrient contents of wheat plants, improving the concentrations of P, K, N, Ca, Mg, Na, Cu and Ni but reducing the Mn and Fe contents in shoot part of plants. The increase of N, Cu, Mn and Ni were observed in roots of M plants in contrast to NM wheat plants. In comparison to shoot part, increased nutrient contents N, Ca, Na, K and Ni were observed in roots tissue of wheat under Zn applied levels (Table 2).

Phosphorus (P) content in plant tissues

The results of Figure 3a and b indicate that significantly higher P contents were observed in M associated plants at 100 and 300 mgkg^{-1} of applied Zn concentration in soil. However, lower P contents were observed at highest applied Zn concentrations (900 mgkg^{-1}) in M treatments. It was observed from the results of this study, that the shoots of M associated plants accumulated higher P level than roots at lower (100 mgkg^{-1}) and moderate (300 mgkg^{-1}) applied Zn levels. At highest applied Zn concentrations (900 mg kg^{-1}), P contents were significantly lower in both M and NM plants.

Table 2. Concentration of micro-nutrients measured in shoots and roots of mycorrhizal (M) and non-mycorrhizal (NM) wheat plants grown in soils with increasing Zn concentrations.

Experiment (Zn, mgkg^{-1})		Cu (mgkg^{-1})	Mn (mgkg^{-1})	Fe (mgkg^{-1})	Ni (mgkg^{-1})
Shoot					
0	NM	9.7200±0.7514^{c}	167.77±15.061^{ab}	55.880±2.8630^{bc}	11.900±1.4619^{abc}
	M	16.253±0.6567^{a}	132.76±7.3253^{bc}	94.800±2.3908^{a}	7.7500±1.0707^{c}
100	NM	11.950±0.9110^{bc}	171.11±19.766^{ab}	45.080±5.5245^{c}	15.800±0.6799^{a}
	M	18.250±0.4819^{a}	113.62±10.174^{c}	64.533±4.3521^{b}	9.3967±0.5584^{bc}
300	NM	12.127±0.9167^{bc}	135.67±7.8387^{bc}	46.023±4.2411^{bc}	9.7033±0.2510^{bc}
	M	18.960±0.9349^{a}	91.437±6.5882^{c}	53.063±4.0966^{bc}	8.6000±0.3126^{bc}
900	NM	15.547±0.4620^{ab}	170.40±8.7727^{ab}	39.260±3.1892^{c}	14.607±1.2584^{a}
	M	17.360±1.1837^{a}	192.92±1.8018^{a}	50.547±4.0839^{bc}	12.763±0.7354^{ab}
Roots					
0	NM	10.947±0.7717^{cd}	52.057±1.1984^{cd}	30.273±2.8794^{b}	5.3300±0.5508^{b}
	M	16.780±0.8600^{ab}	49.810±4.0093^{d}	37.590±4.0771^{ab}	4.6867±0.2730^{b}
100	NM	8.8067±1.1851^{d}	76.147±4.2048^{ab}	49.987±3.2701^{a}	5.9833±0.3262^{ab}
	M	12.920±0.8905^{bcd}	56.410±3.1158^{cd}	50.997±4.2143^{a}	4.7100±0.3635^{b}
300	NM	8.1567±0.8442^{d}	80.147±2.6444^{ab}	42.000±4.8182^{ab}	6.1733±0.1048^{ab}
	M	14.067±1.7152^{bc}	67.233±2.6548^{bc}	46.510±2.3832^{ab}	5.1467±0.2896^{b}
900	NM	12.347±0.4798^{bcd}	86.947±5.2867^{a}	35.877±2.9880^{ab}	7.0833±0.7714^{ab}
	M	20.917±0.7835^{a}	90.123±3.6968^{a}	41.640±4.6277^{ab}	8.3667±0.8604^{a}

Data are presented as mean ± SD (n = 3) and have been analyzed by two way analysis of variance. Means followed by the same letter within columns are not significantly different by Tukey's test at the 5% level.

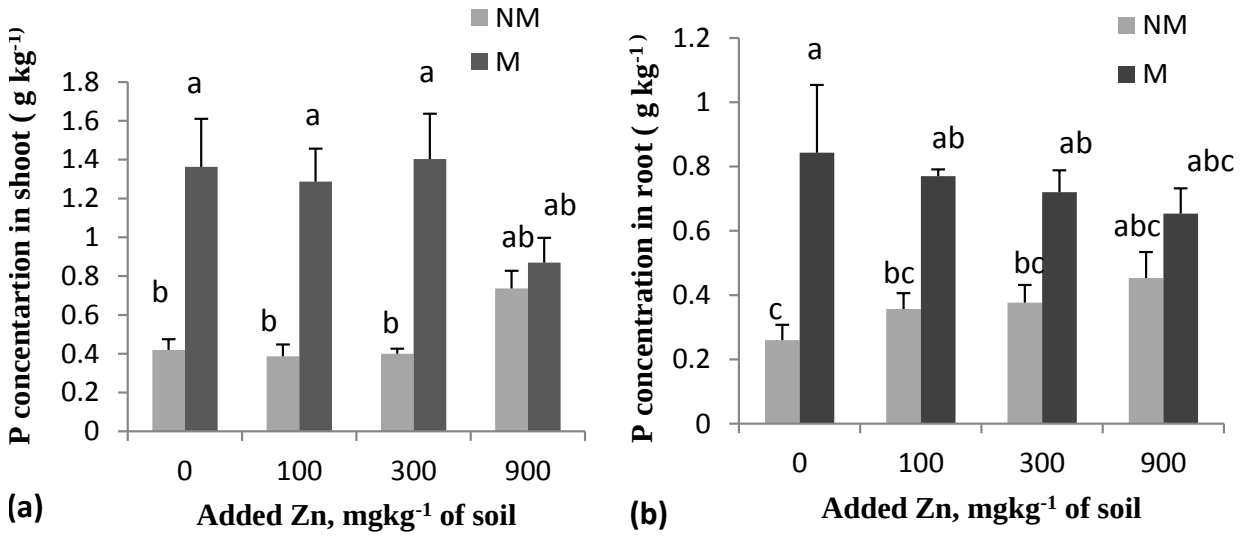

Figure 3. Phosphorus concentration in (a) shoots and (b) roots of mycorrhizal (M) and non-mycorrhizal (NM) wheat plants in response to Zn addition to soil. Means (n = 3) with the different letters are significantly different (*P< 0.05*) by the Tukey test.

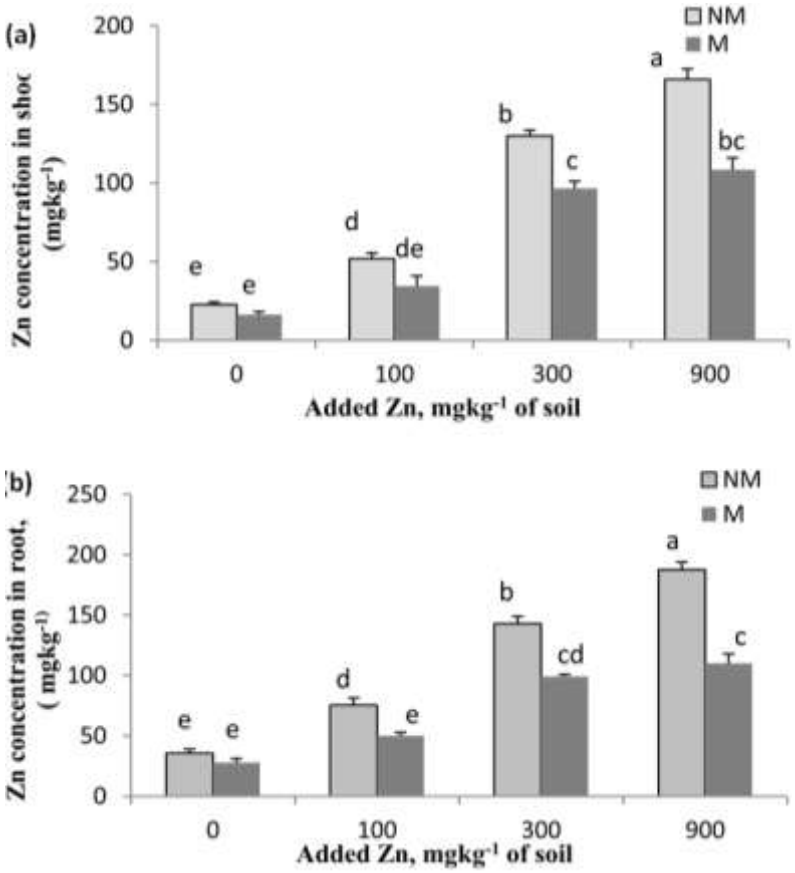

Figure 4. Zn concentrations in (a) shoot, (b) root of mycorrhizal (M) and non-mycorrhizal (NM) wheat plants grown in soil with increasing Zn concentrations, respectively. The different letters above the bars indicate significant difference between treatments. Bars represent standard error; M, black dots, NM, light grey dots.

Zinc uptake in plants tissues

Figures 4a and b shows the uptake of Zn in shoots and roots of plants in both M and NM associated wheat plants at each applied Zn concentrations. The results indicate that Zn contents increased in plants tissues as the concentration of Zn was increased in the soil. In NM treatments, more Zn uptake was recorded in wheat tissues (shoot and root) as the Zn contents increased in soil (100, 300 and 900 $mgkg^{-1}$).

When no Zn was applied (0 application level), the non-significant results were observed in both M and NM treated plants. The results show that in NM plants, as the Zn concentration increased in the soil, more Zn was accumulated in shoot part as compared to root part of plants. The higher Zn concentration was observed at 900 $mgkg^{-1}$ in both M and NM treated plants.

Biochemical contents in plant tissues

Figure 5a to c shows the chlorophyll and carotene of M and NM wheat plants. The results show that in M plants, the chlorophyll (a, b) and carotene contents (c) observed were significantly higher in contrast with NM associated wheat plants at all applied Zn concentrations (0, 100, 300 and 900 $mgkg^{-1}$). At Zn concentration of 100 $mgkg^{-1}$, the more chlorophyll a and b were recorded in both treatments. At highest Zn concentration (900 $mgkg^{-1}$), lowest chlorophyll a and b contents were observed in M and NM associated wheat plants.

Figure 5d shows content of proline in M and NM inoculated wheat plants as the applied Zn concentration increased (0, 100, 300 and 900 $mgkg^{-1}$) in soil. It was observed from the results of present study that proline contents were significantly increased as the concentration of Zn increased in the soil. The results show that proline content was higher in NM plants as compared to M plants but the trends observed were same at all applied Zn concentrations.

Figure 5e shows contents of sugar in M and NM treatments under increasing Zn levels in soil. The results indicate that M plants showed significantly higher sugar contents as compared to NM plants tissues. Total sugar

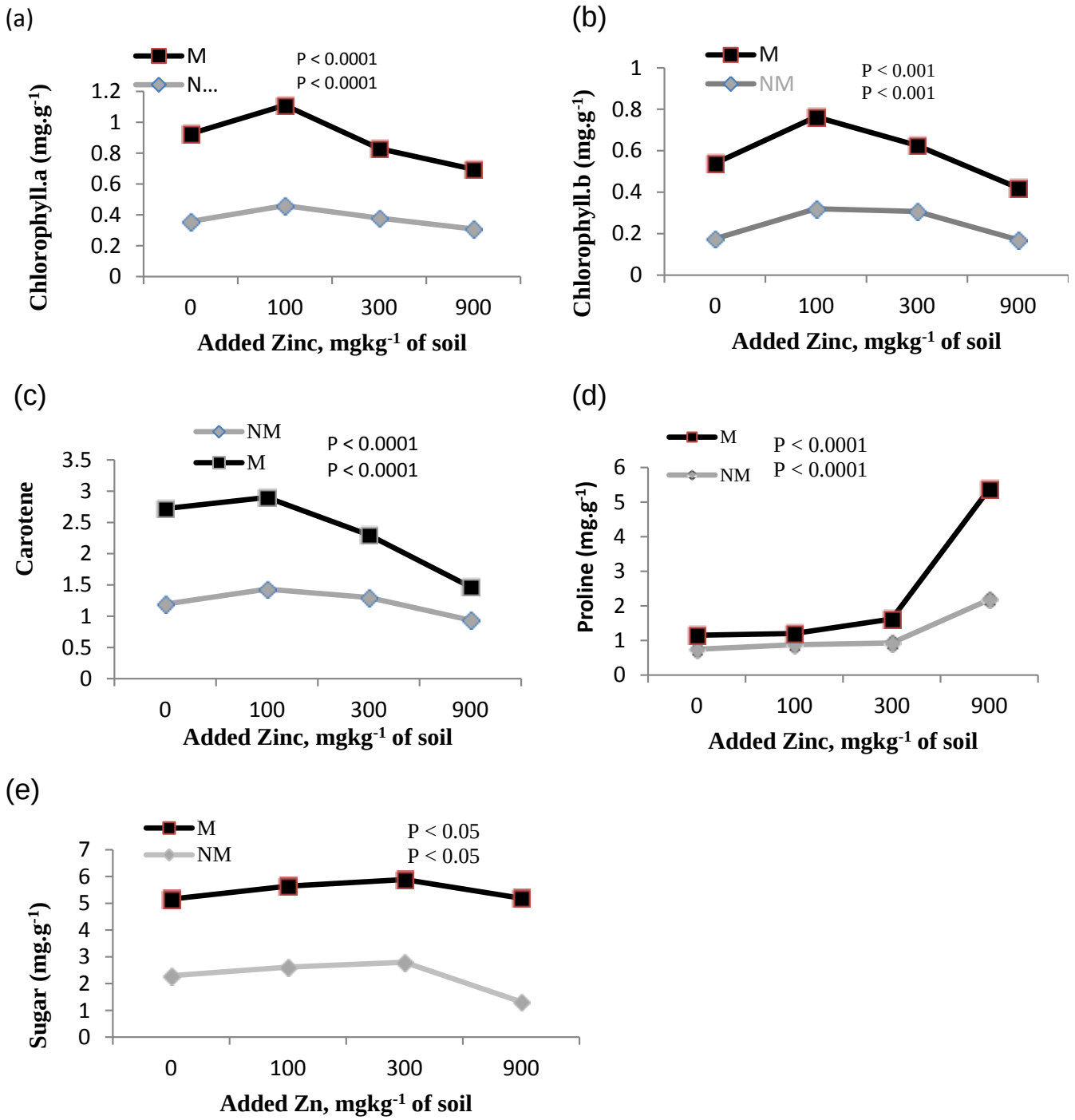

Figure 5. Biochemical contents. **(a and b)** Chlorophyll a, b content. **(c)** Total carotene content. **(d)** Proline contents. **(e)** Sugar contents in leaves of mycorrhizal (M) and non-mycorrhizal(NM) wheat plants with increasing Zn concentrations in soil (*P< 0.05* significant by the Tukey test (5%) for M and NM means for each Zn concentration).

contents were improved in plants shoot and roots as the Zn concentration increased in soil (100 and 300 mgkg^{-1}). The decrease of sugar content was observed at highest applied Zn level (900 mgkg^{-1}) in both M and NM associated wheat plants.

Antioxidant enzyme activities in plants

Figure 6 shows the trends of antioxidant enzymes (SOD, CAT, APX, POD) activities in shoot and root of both M and NM associated wheat plants under increasing Zn

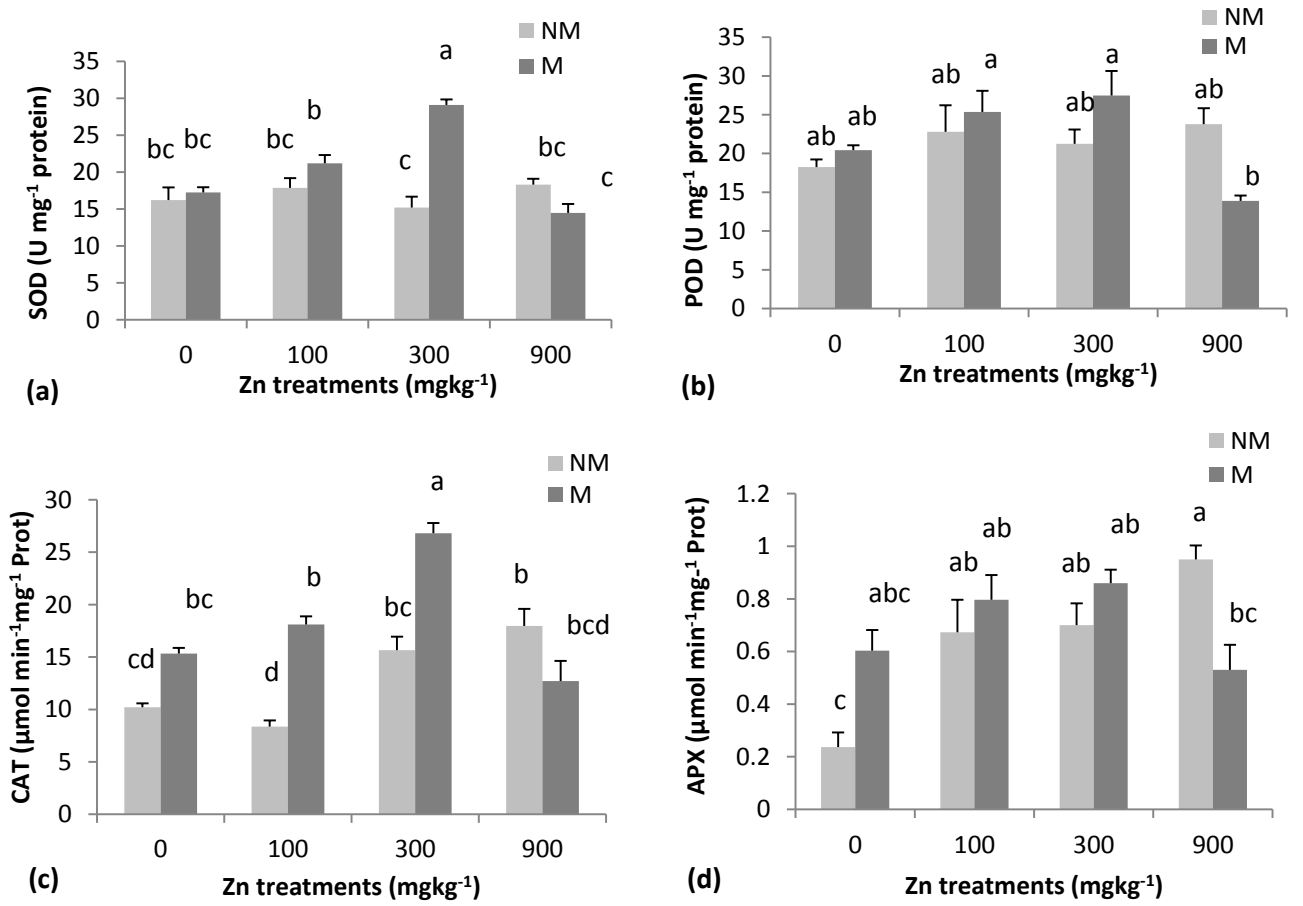

Figure 6. Antioxidant enzymes activity. **(a)** SOD activity. **(b)** POD content. **(c)** CAT activity. **(d)** APX activity, in leaves of mycorrhizal (M) and non-mycorrhizal (NM) wheat plants in response to Zn addition to soil. Means (n = 3) with the different letters are significantly different ($p < 0.05$) by the Tukey test. Bars represent standard error.

concentrations (0, 100, 300 and 900 mgkg^{-1}). Figure 6a shows that in NM associated treatments, the contents of SOD was low as compared to M plants and a slight increase in concentration was observed as more zinc concentration was introduced in soil. The highest activity of SOD in M plants was observed at 300 mgkg^{-1}.

Figure 6b shows M inoculated plants; Zn exposure causes a significant reduction of POD content at Zn concentration of 900 mgkg^{-1}. Figure 6c shows CAT activity improved as the concentration of Zn increased in both NM and M treated plants. At higher Zn application level (900 mgkg^{-1}), the decrease CAT activity was observed. Figure 6d shows APX activity was enhanced as the Zn application increased in soil. The observed trends were same for both APX and CAT in M treated plants.

DISCUSSION

AMF can reduce Zn toxicity in plant species and affects positively on growth of plants in comparison to non-AMF plants (Arriagada et al., 2005). The results indicate that AMF inoculated wheat plants with different Zn concentrations showed increased plant growth than NM treated plants. However, Zn concentrations >300 mg/kg may inhibit root and shoot growth showing Zn phytotoxicity. Zn is an essential nutrient but more concentration of Zn (< 300 mg/kg) can decrease plant growth and in some cases cause inhibition of plant growth (Gratao et al., 2005). This might be due to the fact that less Zn concentration can cause inhibition of plants for further growth. The other possible reason is more pH of soil (7.8) may cause Zn availability to plant tissues which become immobilized and reduce its uptake to shoot and roots.

As Zn concentration increased in soil, percentage of mycorrhizal colonization in roots of wheat plants was significantly reduced. Several authors described that the reduction of mycorrhizal colonization with plants roots is due to increase toxicity of heavy metals. Some studies reported that mycorrhizal colonization do not decrease in plants growing with high metal contents (Li et al., 2011). This might be due to the fact that species of mycorrhiza

isolated from heavy metals polluted soil are more tolerant and resistant to metals as compared to the species that are less tolerant (Leung et al., 2006).

In the present study, Zn uptake was increased in plants shoots and roots at lowest applied Zn levels and the defensive mechanism of mycorrhizal fungi in plants tissues with higher Zn concentrations were recorded. NM treated plants showed higher Zn concentrations in their shoots which cause reduction of plants yield. AMF fungus protects the plants from moderate Zn contamination by immobilizing Zn in the fungal mycelium. This may be due to the mechanism that mycorrhizal plants might lower the uptake of heavy metals contents. Mycorrhizal association also increased uptake of important nutrients especially P. The increase of P content and other nutrients such as N, Mg, Ca and K in M plants also enhanced the productivity of plants (Chen et al., 2003). The improvement of P contents in AMF associated plants is due to its increase uptake through mycorrhizal structures, that is, arbuscules and vesicles (Smith and Read, 1997).

The significant nutrients deficiency was recorded in NM treated wheat plants grown in soil with increased Zn concentrations as compared to M treated plants. The reduced K uptake in plant shoot was observed as the Zn concentrations increased in NM treated wheat plants. This phenomenon might be due to removal of K in roots at higher Zn concentrations and also due to membrane rupture of plants that cause K leakage (Bonnet et al., 2000).

The increased metals toxicity also increase proline contents and different amino acids as the metals concentrations increased in the soil. This can be the protective mechanism of plants tissues under high metals stress conditions. One mechanism of proline is to decrease the free radicals production as the metals concentrations increased in soil (Mittler, 2002). The increase of proline contents in plants shoot is also one of the indication of increased metal toxicity. Antioxidant enzymes (CAT, APX and SOD) play a vital role in increasing defensive mechanisms towards more ROS production (Foyer et al., 2005). It is suggested from the results of present study that AMF is able to maintain mycorrhizal symbiosis in Zn toxic soils and significantly increase the plant growth, productivity and nutrient contents.

Conclusion

The study concluded that inoculation of mycorrhizal fungal species in plants had useful impacts on increasing the growth, biomass, yield, and nutrient contents of plants. Better growth, biomass, improved nutrients uptake and higher yields were recorded in M treated plants as compared to NM treated plants. However, in M plants, the Zn contents were lower in shoot at the highest applied Zn levels (900 $mgkg^{-1}$). This decrease of Zn transfer from soil to shoot tissue shows the ability of AMF inoculum to alleviate metal toxicity in wheat plant. In general, AMF protected the wheat plants against metal toxicity and also improved the nutrients contents of plants. The importance of mycorrhization for wheat plants under high Zn concentrations was observed. As levels of heavy metals is increased due to increase use of sewage sludge in agriculture, and also due to industrial emissions that cause to contaminate the agricultural soils. The useful application of mycorrhizal association for cereal crops in field can improve agricultural ecosystem. Furthermore, experiments under field conditions should be employed to study the extent to which mycorrhizal fungi can alleviate Zn plant toxicity.

Conflict of Interests

The authors have not declared any conflict of interests.

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Table S1. Chemical characteristics of soil samples used for growing of wheat plants in pot experiment.

Parameter analyzed	Concentration ($mgkg^{-1}$)
T. Phosphorus (P)	4.3
T. Potassium (K)	19.5
Calcium (Ca)	34.45
Magnesium (Mg)	42.50
Extractable nitrate nitrogen (NO_3-N)	1.04
Extractable potassium (ext-K)	1.45
Extractable phosphorus (ext-P)	1.53
Zinc (Zn)	1.50
Nickel (Ni)	1.33
Copper (Cu)	30.3
Cadmium (Cd)	1.60
Iron (Fe)	28.51
Lead (Pb)	1.6
Chromium (Cr)	4.25
Manganese (Mn)	10.4

Data represents the average concentrations of macro and micronutrients in the soil used in pot experiment.